Composition Studies on Tobacco XXIV. High Molecular Weight Pigment In Smoke of Various Tobaccos

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Recently a high molecular weight pigment was isolated from cigarette smoke (4). The properties of this pigment were similar to certain polymeric substances composed of amino acids, polyphenols, and iron, which had been reported previously in tobacco leaf (2, 3, 5). In a continuation of this work, a survey has been completed of the levels and other characteristics of the pigment in smoke condensates from various blended and unblended cigarettes.

Method

All cigarettes were custom made on a commercial production-line machine, were 85 mm. in length without filters or additives and were equilibrated to yield moisture contents of 12-14% before smoking (1). Five types of cigarettes were studied, four unblended (straight flue-cured, burley, Maryland, and Turkish) and one blended (40% flue-cured, 35% burley, 5% Maryland, and 20% Turkish). The cigarettes were smoked on an automatic smoking machine to an average butt length of 25 mm. using the following conditions: puff volume, 35 ml; puff duration, 2 sec.; puff rate, one per min. The condensate was collected in glass traps cooled in a Dry-Ice acetone mixture and was removed from the traps with acetone after which the acetone solution was evaporated to dryness *in vacuo*.

The procedure for isolation of the pigment is shown in **Fig. 1**. In each case, condensate from 510 cigarettes was partitioned between equal volumes of ether and 1N NaOH (5 ml each per gram condensate) and the layers were separated. The aqueous layer was diluted 8-fold with water and slowly acidified to pH 3.2 with H_2SO_4. Dilution with water was necessary to obtain a brown flocculent precipitate of pigment instead of the black tar obtained without extensive dilution. The precipitate was washed thoroughly with water, redissolved in 1N NaOH and reprecipitated as above. The filtered pigment was washed thoroughly with water and dried over calcium chloride. The dried precipitate was then continuously extracted with ether for 32 hours. The ether-insoluble pigment was then dried over calcium chloride for 96 hours and weighed.

Molecular weight approximations were made by gel filtration through columns of polyacrylamide (Bio Rad Laboratories).[2] The pigment samples were suspended in distilled water and the pH was adjusted to 12.0. After thorough mixing, the pH was dropped to pH 10 by the addition of dilute HCl after which the mixture was diluted with concentrated phosphate buffer (pH 10.0) to yield a final concentration of 0.01 M phosphate and was filtered prior to addition to the column. About 120 mg. of pigment per 100 g. of gel was used and all columns were eluted with 0.01 M phosphate buffer (pH 10.0).

Results and Discussion

The method was intended for use merely in a superficial survey of the various smoke condensates rather than a precise analytical procedure. Methodological studies on commercial cigarettes showed an approximate range of values of ± 10% in replicates. Values obtained for the various condensates are given in **Table 1** and are single determinations except for the burley condensate, which was confirmed.

The levels of pigment in all condensates are comparatively high, indicating that the substance must be considered a major constituent of smoke from cigarettes of all types. On the basis of weight of pigment per cigarette, values from about 1.0 to almost 3.0 mg. were obtained. Among the unblended types, smoke from Turkish and Maryland cigarettes contained the larger amounts. On the basis of pigment weight per gram of tobacco smoked, condensate from Maryland cigarettes gave a higher value than Turkish, which result is a reflection, at least in part, of the larger specific volume of the former tobacco type. In all cases the yield from burley tobacco smoke was the lowest of all types. The weight of pigment per cigarette for the blend was calculated to be 119% of the theoretical based on an assumed additive effect of the various tobacco types present therein.

The patterns of molecular weight distributions in the various pigment by gel filtration are given in **Table 2**. In each case the major band was

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[2] *Use of a commercial product does not constitute endorsement by the U. S. Department of Agriculture over other products of a similar nature.*

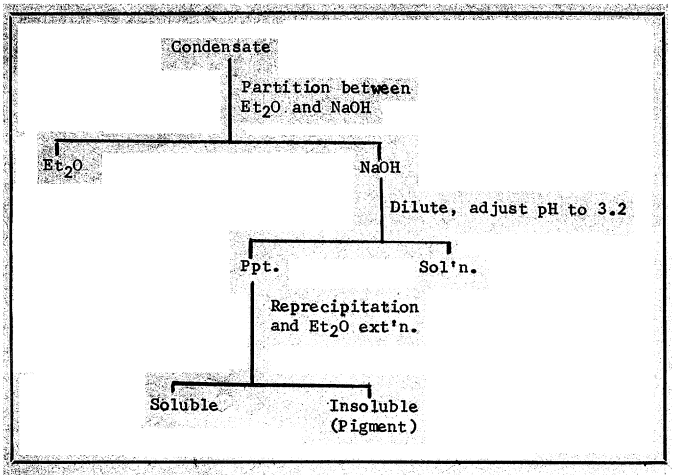

Figure 1. Scheme for separation of smoke pigment.

Table 1. Pigment levels in smoke

	Pigment wt. (mg.)		
	Per cigarette	Per g. tobacco smoked	Per g. condensate
Flue-cured	1.87	1.97	42
Burley	1.08	1.56	30
Maryland	2.71	4.62	50
Turkish	2.72	2.55	42
Blend	2.15	2.53	52

determined by color intensity on the column and in the eluates. Although indicated as "major" and "minor" in the table, some variability was obtained with the flue-cured pigment and it is best to state that the bands were probably of equal intensity. The major band of the burley pigment had a molecular weight much lower than the major components of the other types. The blended cigarettes had values expected for a mixture of the four constituent types. The major, high molecular weight band in the blended cigarettes is apparently contributed by the flue-cured and Turkish tobaccos and the minor band by the burley. The values obtained for these blended cigarettes closely approximated previous results for the pigment of commercial blended cigarettes (4). All smoke condensates also contained fractions having molecular weights between the limits indicated in Table 2 but these appear to be minor ones which could not be resolved.

Turkish, Maryland, and burley pigments contained some material which was insoluble in alkali at pH 12.0 during preparation of the pigment solution for gel filtration. Based on the total pigment isolated, the following amounts of alkaline-insoluble pigment were obtained: Burley, 35%; Maryland, 7%; and Turkish, 16%. Flue-cured pigment contained little or no insoluble material and the blend was completely soluble. The origin of this insoluble material is not known. An obvious possibility is structural alteration on handling although there was no other indication of this during the work. In all cases only the alkaline-soluble material was subjected to gel filtration.

Summary

Levels of the high molecular weight smoke pigment previously shown to contain chlorogenic acid, amino acids and iron were determined in condensates from blended and unblended cigarettes. Also, approximations were made of the molecular weight distributions of the subfractions of the pigments in the condensates. Burley pigment was grossly different from the other types in that the molecular weight of the major fraction is much lower and a relatively large amount becomes insoluble in alkaline solution of pH 12.0 after purification. Turkish and Maryland condensates contained higher weight levels of pigment per cigarette and burley contained the lowest level.

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Table 2. Approximate molecular weights of pigment subfractions

	Major band	Minor band
Flue-cured	70,000-80,000[a]	1,500[a]
Burley	4,500-5,000	10,000-20,000
Maryland	40,000	$\leqslant$ 5,000
Turkish	$\geqslant$ 100,000	1,000
Blend	80,000-90,000	$\leqslant$ 10,000

[a] *May be of equal intensity.*